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Hosted By:
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Confined Placental Mosaicism In Infants with Fetal Growth Restriction

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Confined Placental Mosaicism - Historical Perspective

- occurs in 1-2 % of first trimester CVS samples
- all chromosomal mosaicism in placental samples is not confined
- 1/3 represents true mosaicism

Etiologies

- Post fertilization event confined to one cell line
- “Rescue” to diploidy of an originally trisomic conception

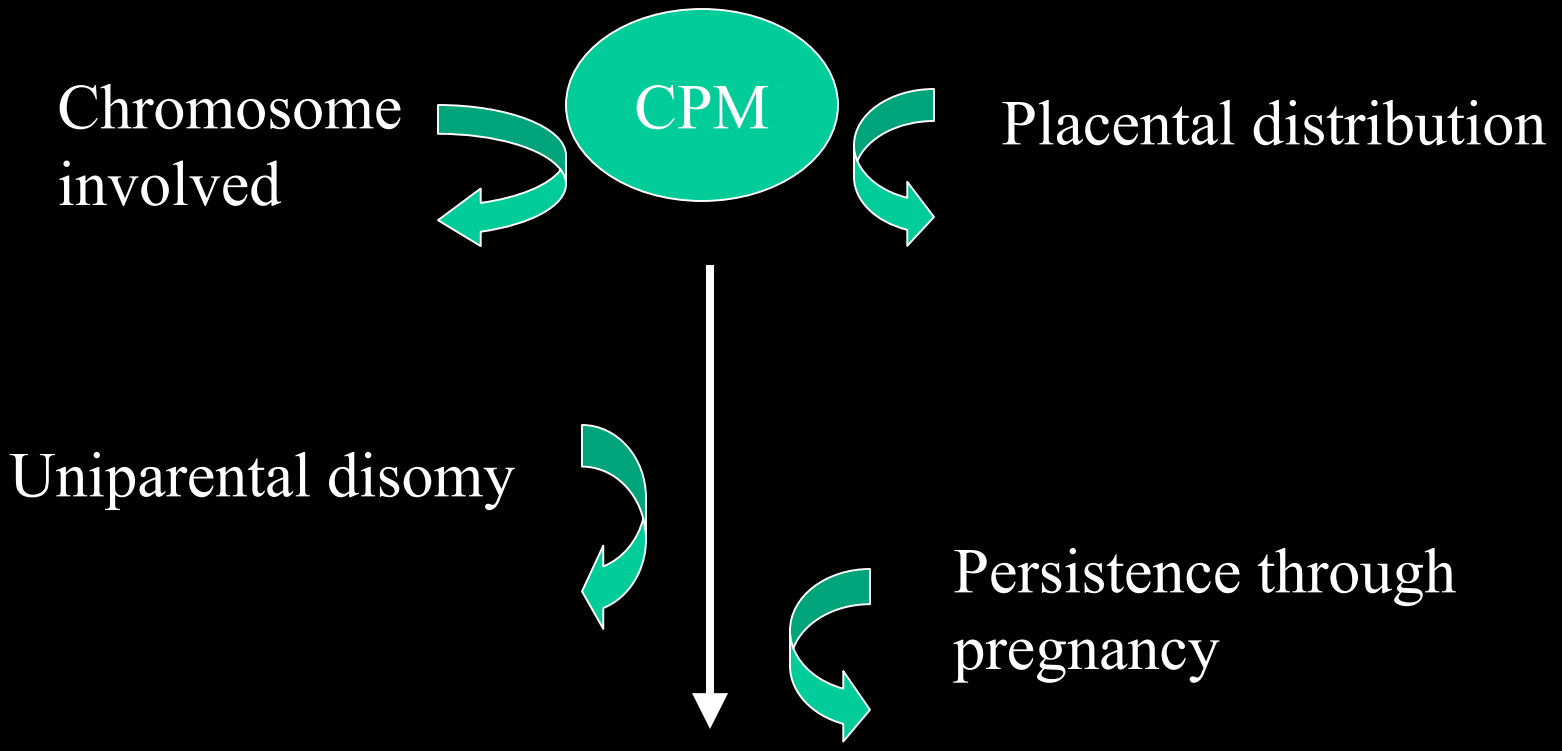
Approaches to the study of CPM and fetal growth restriction

- Cohorts with CPM identified first trimester (CVS)
- Cohorts of newborns
- Case control studies of newborns

Approach : follow-up of cohorts with CPM diagnosed first trimester

- Adverse outcomes suggested over 10 years ago – pregnancy loss, stillbirth, growth restriction

Variation in outcomes



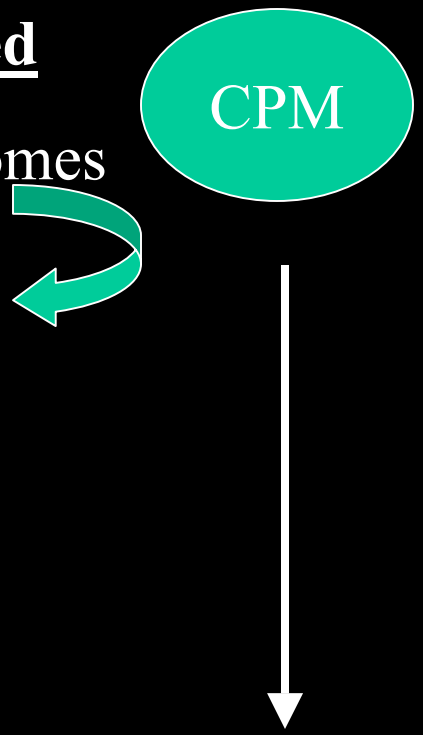
Variation in outcomes

Chromosome involved

2,3,7,8 normal outcomes

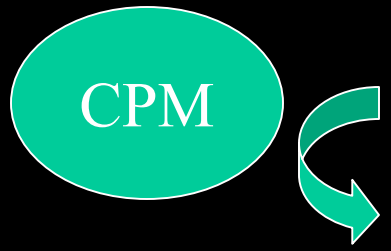
9, 16, 22 of meiotic origin associated with IUGR

(Robinson, 1997)



FETAL GROWTH RESTRICTION

Variation in outcomes



Placental distribution
(Kalousek, 92; Simoni, 1994)

Type I – cytoptrophoblast
direct preparation
Type II – extraembryonic mesoderm
culture preparation
Type III – both cell lines

FETAL GROWTH RESTRICTION

Variation in outcomes

Uniparental disomy
(Robinson, 1997)

CPM16	IUGR	normal
Fetal UPD	11	2
Fetal BPD	5	8

FETAL GROWTH RESTRICTION

Variation in outcomes

<u>Persistence through pregnancy</u>

-variable 50-80%

-35% rate of IUGR

(Kalousek, 1991)

FETAL GROWTH RESTRICTION

Approach : CPM among a cohort of newborns (Artan, 1995)

- Karyotypes from 125 term placentas of pregnancies delivered following prenatal determination of normal fetal karyotype (AMA indication)
  - Higher risk population for nondisjunction
  - 6/125 (4.8%) CPM

- All 6 cases of CPM ended in IUGR infants
  - 46,XX/47,XX,+14 (125/25) 2414 39 wks
  - 46,XX/92,XXXX (74/76) 1647 34 wks
  - 46,XY/47,XY, +21 (124/26) 2100 36 wks
  - 46,XX/47,XX,+21 (73/87) 2400 40 wk
  - 46,XX/45,X (61/79) 1760 38 wk
  - 46,XY/47,XY,+18 (61/79) 2200 39 wks
- Birthweights CPM=2086+/-131.5;
  - normal placental biopsies 3305.2+/- 28.8

Approach : analysis of growth restricted newborns - Unanswered Questions

- How large of a contributor is CPM to the population of infants with growth restriction?
- Are there characteristic clinical findings?

Study Proposal for Case/control analysis– Primary Aim

- Determine the frequency of CPM by karyotype analysis of placental biopsies from infants with growth restriction compared to biopsies from placentas of maternal age matched, appropriately grown infants

Study Proposal – Secondary Aims

- Utilize molecular, chromosome specific polymorphisms to identify uniparental disomy or low level mosaicism in a subset of patients if CPM not identified cytogenetically
- Explore clinical variables for identifying characteristics

Background

- Which IUGR populations have been studied?
- Which chromosomes? Tetraploidy ?
- Alternative ways to search
  - Traditional cytogenetics
  - Molecular cytogenetics (FISH)
  - Molecular genetics (dinucleotide repeats)

Studies of infants with unexplained IUGR

• Kalousek, 1983	2/9
• Verp, 1990	0/11
• Krishnamoorthy,1995	4/26
• Wilkins-Haug, 1995	3/12
• Cowles, 1996	1/20
• Stipolijev, 2001	3/20
	13 / 98 (13.2 %)

CPM among different populations of IUGR infants

Kennerknect, 1993

- Newborns presenting with SGA 0/71
- Newborns having normal CVS
  - who developed SGA (24/1300) 5/24
- Controls 0/20

What do these studies suggest?

- CPM may play a role in the significantly IUGR population – those characterized by antepartum diagnoses, nonreassuring fetal well-being
- Sample sizes of both case and controls need to be adequate
- Role of tetraploidy ?

Aneuploidy versus tetraploidy – Is there any evidence to support tetraploidy as a pathologic factor?

- Considered artifact - time in culture
  Tegenkamp, 1976; Kaji. 1979, 1981)

Does tetraploidy occur “ in vivo”?

- preimplantation embryos
- uncultured amnion by sex chromatin and cellular DNA determinations (Klinger, 1960)
- Tetraploidy by flow cytometry in placenta
  - 2.2% tetraploid

Background rate of tetraploidy

(Noomen, 2001)

- 100 women AMA
- Semi direct and long term culture of chorionic villi
- Up to three tetraploids in 27% of STC
- In all long term cultures

Any association of tetraploidy with abnormal placentation?

- Miscarriages assessed by long term culture (Hunt, 1985)
  - 10-30% in spontaneous miscarriages
  - 10% tetraploidy in first trimester tabs
- Miscarriages assessed by direct preparation (Eiben, 1990)
  - 9.2% tetraploidy

Tetraploidy among CPM

- 5% of CPM is tetraploid mosaic (Ledbetter, 1992)
- ACC UK collaborative data (1994)
  - Tetraploidy noted as well

Materials and Methods

- Antepartum identification of IUGR by ultrasound as <10% for gestational age
- Singleton pregnancies with EDC confirmed by US < 16 weeks gestation
- Excluded maternal conditions of HTN, IDDM, SLE, fetal malformations

Sample Sizes

- 75 IUGR cases without recognized risk factors
- 75 AGA controls matched by maternal age to within 5 years
- 95% confidence with 80% power to detect $\geq$ 15% CPM among IUGR population
- Assumes 0.5 % CPM among AGA controls

Study samples

- placental biopsies
- cord blood for karyotype or ability to recontact
- parental buccal samples or peripheral blood sample for DNA extraction

Placental Samples

- paired chorionic plate samples removed from a mapped 4 locations
- one for culture
- one for disaggregated nuclei (FISH or DNA extaction)

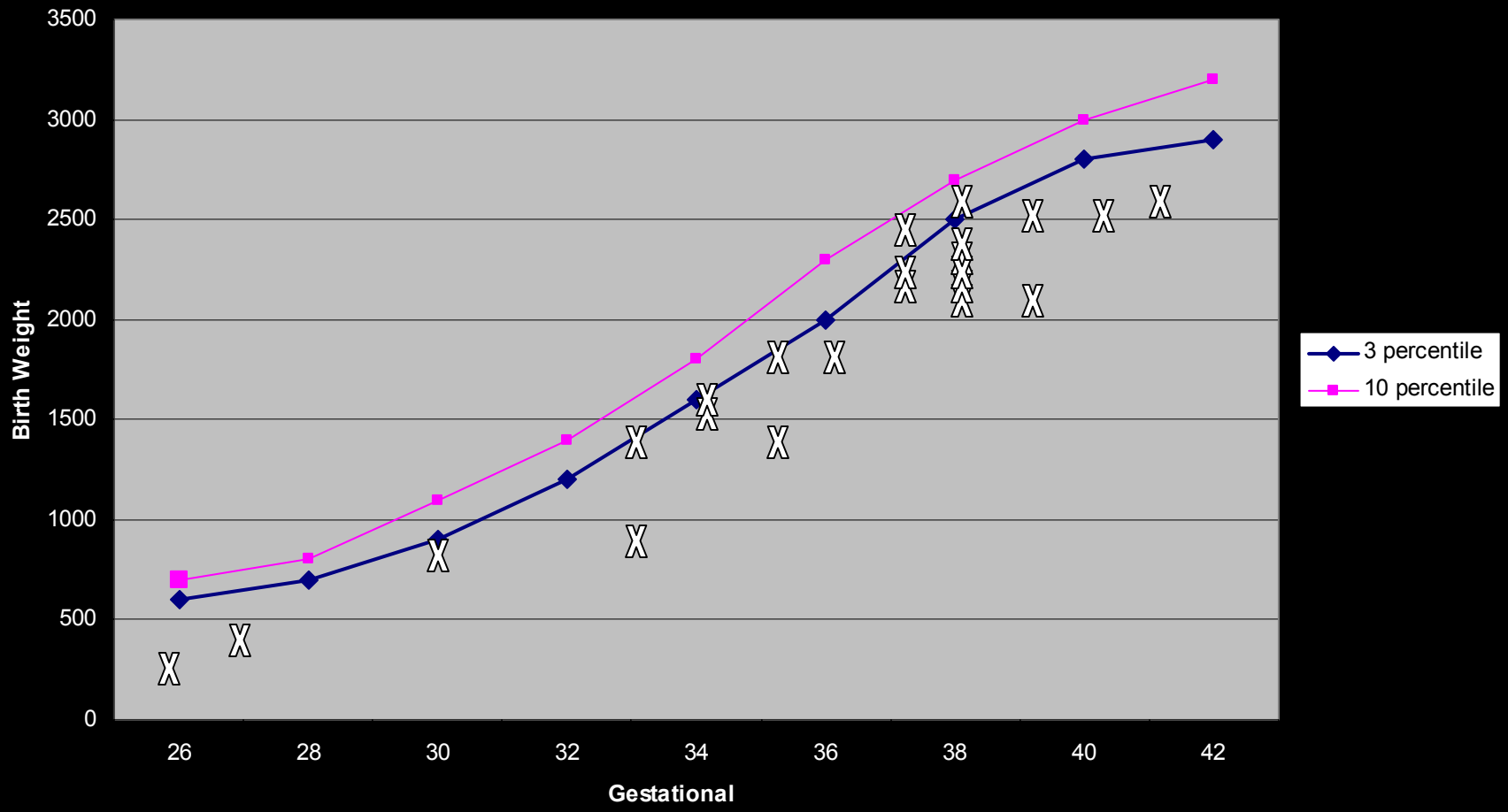
Karyotype analysis

- Cultures established according to routine long term protocols
- 25 cells scored from each site (excludes > 15% mosaicism with 95% confidence)

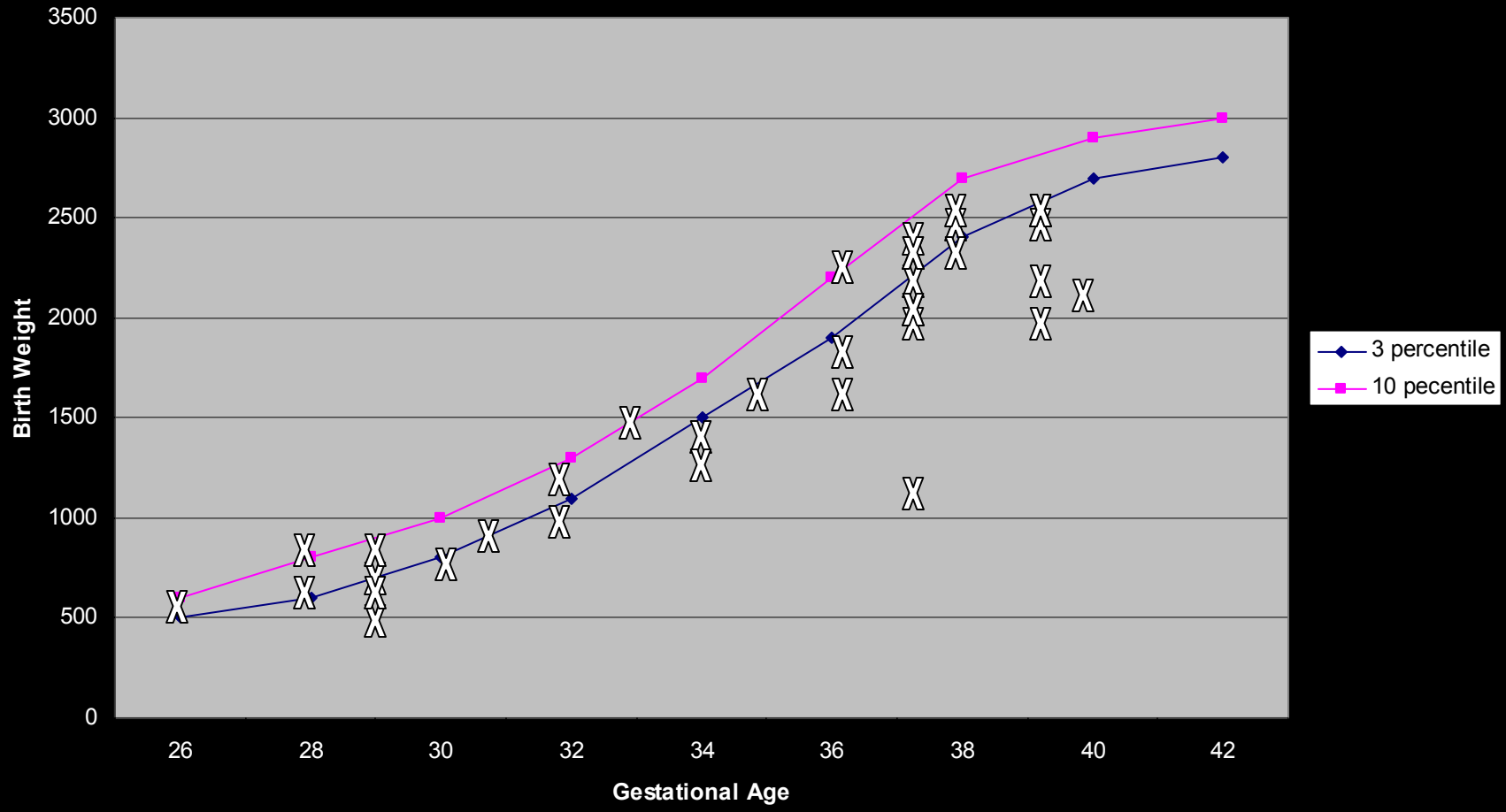
Molecular analysis

- Fluorescent panel of dinucleotide markers with heterozygosity scores of > 0.75
- Automated genotyping on ABI377
- Minimum of 1 and maximum of three markers per each autosome

Birth Weight Distribution - Males



Birth Weight Distribution - Females



Results

	Aneuploid	Tetraploid	Total
• Cases	1	5	6/75
• Controls	1	0	1/75

Aneuploidy Mosaicism

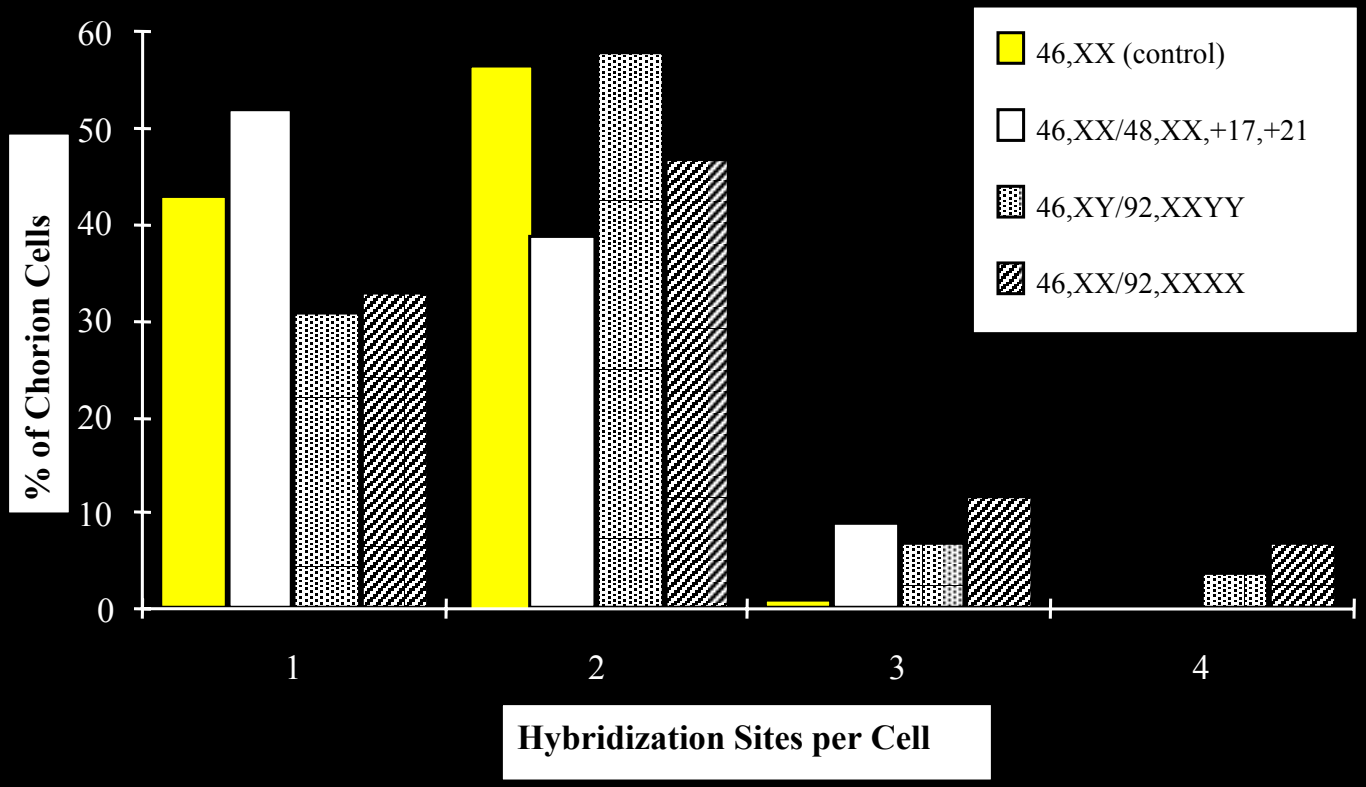
- Case — diploid/aneuploid
  - 46,XX/48,XX,+17,+21 — 14 / 12
- Control
  - 46,XY/47,XY,+10 — 7 / 13

Tetraploid Mosaicism

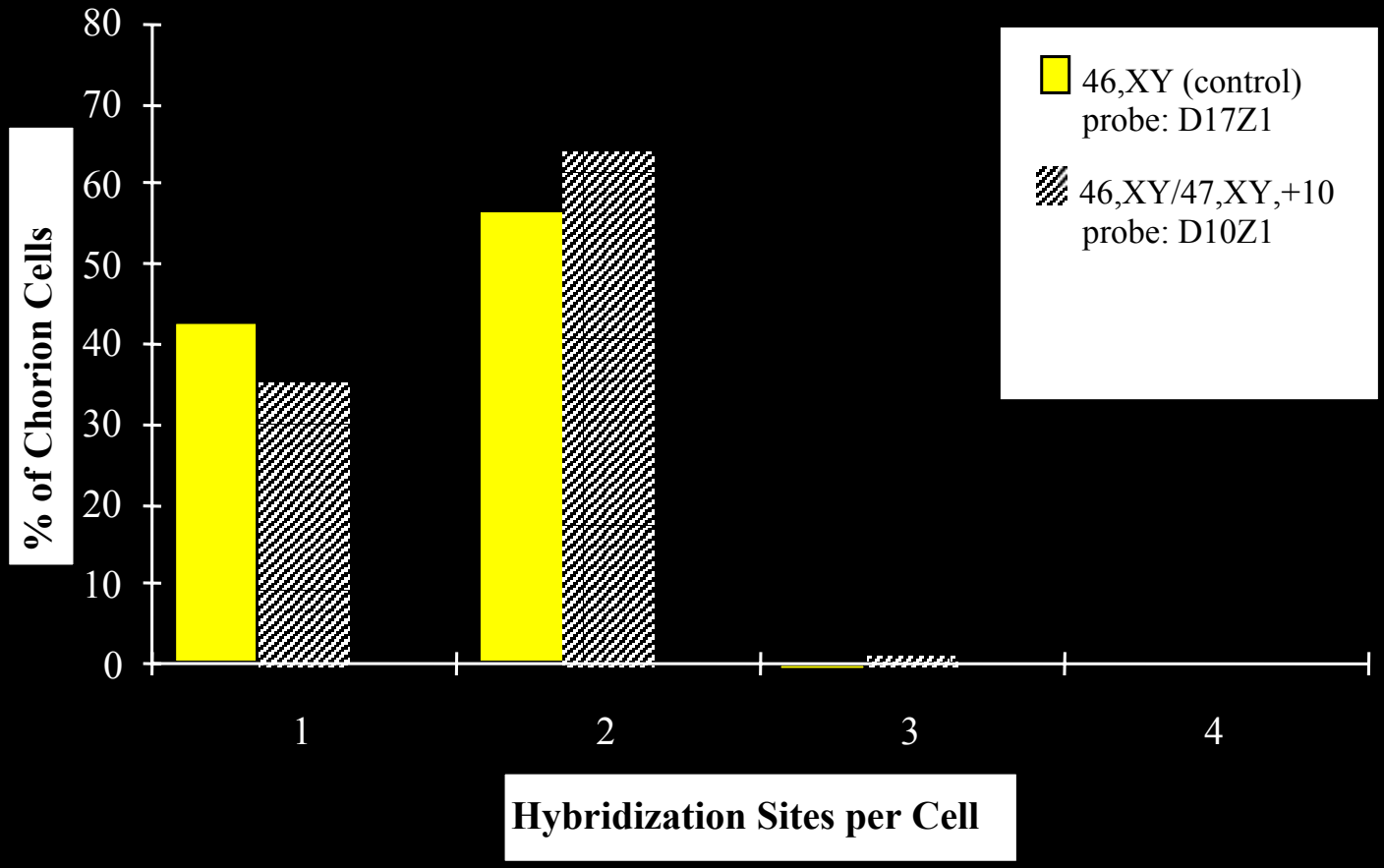
	diploid/polyploid	days in culture
• Cases		
– 46,XX/92,XXXX	25 / 30	10
– 46,XY/92,XXYY	25 / 13	13
– 46,XX/92,XXXX	25 / 23	10
– 46,XX/92,XXXX	25 / 10	8
– 46,XX/92,XXXX	25 / 19	12
• Controls		
– none		

Hybridization Sites in IUGR Placentas

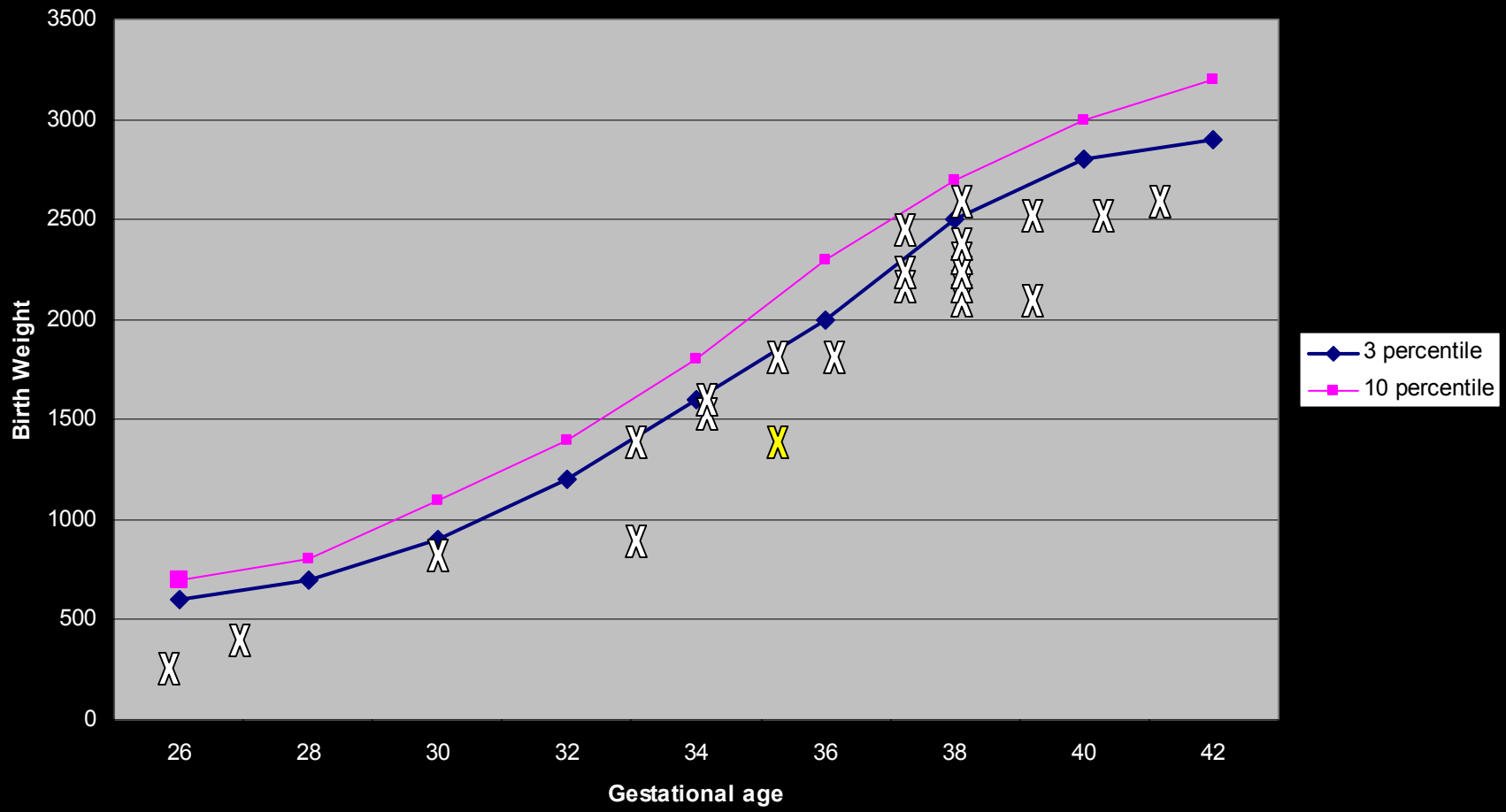
Probe:D17Z1



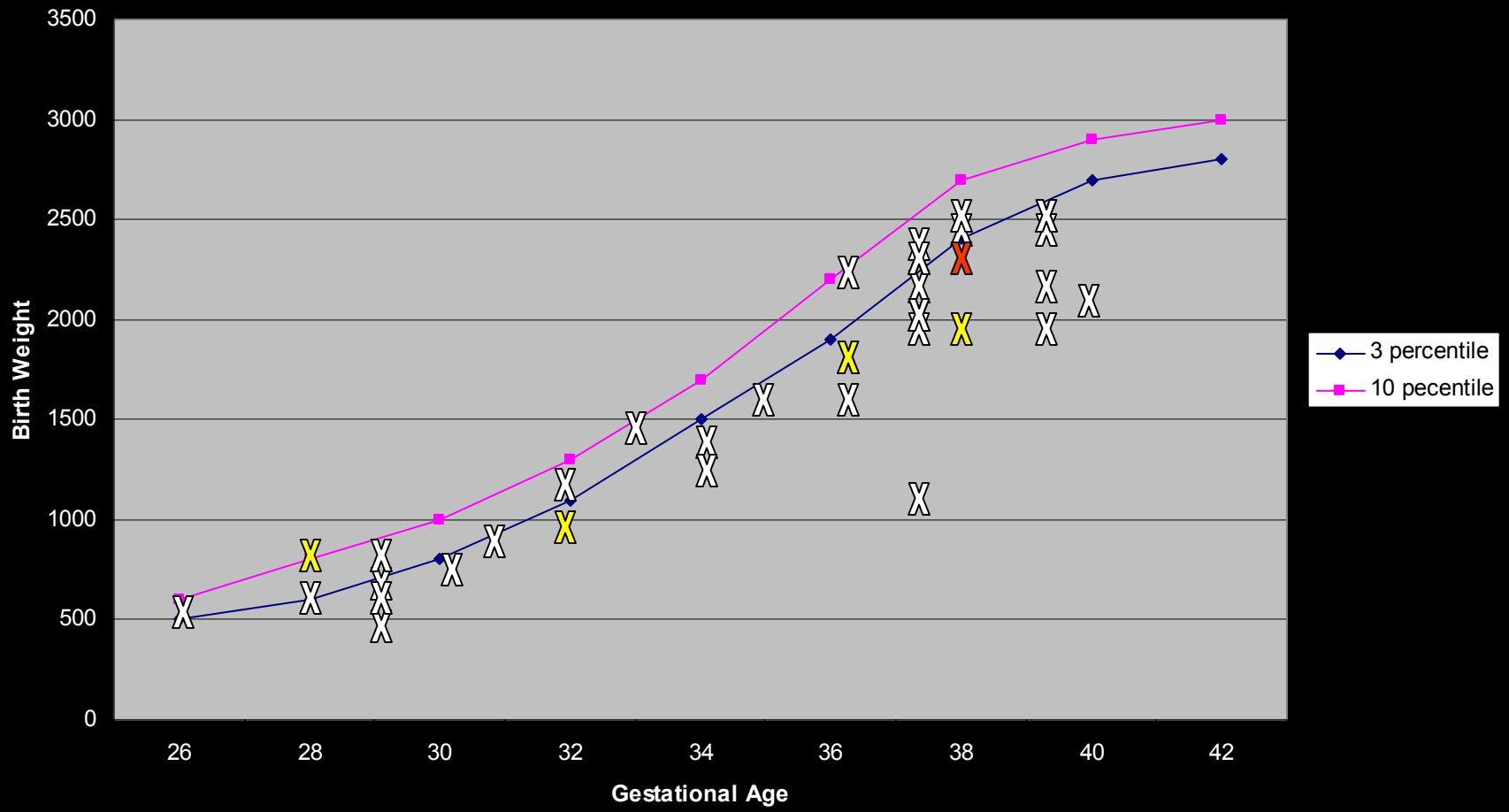
Hybridization Sites in AGA Placentas



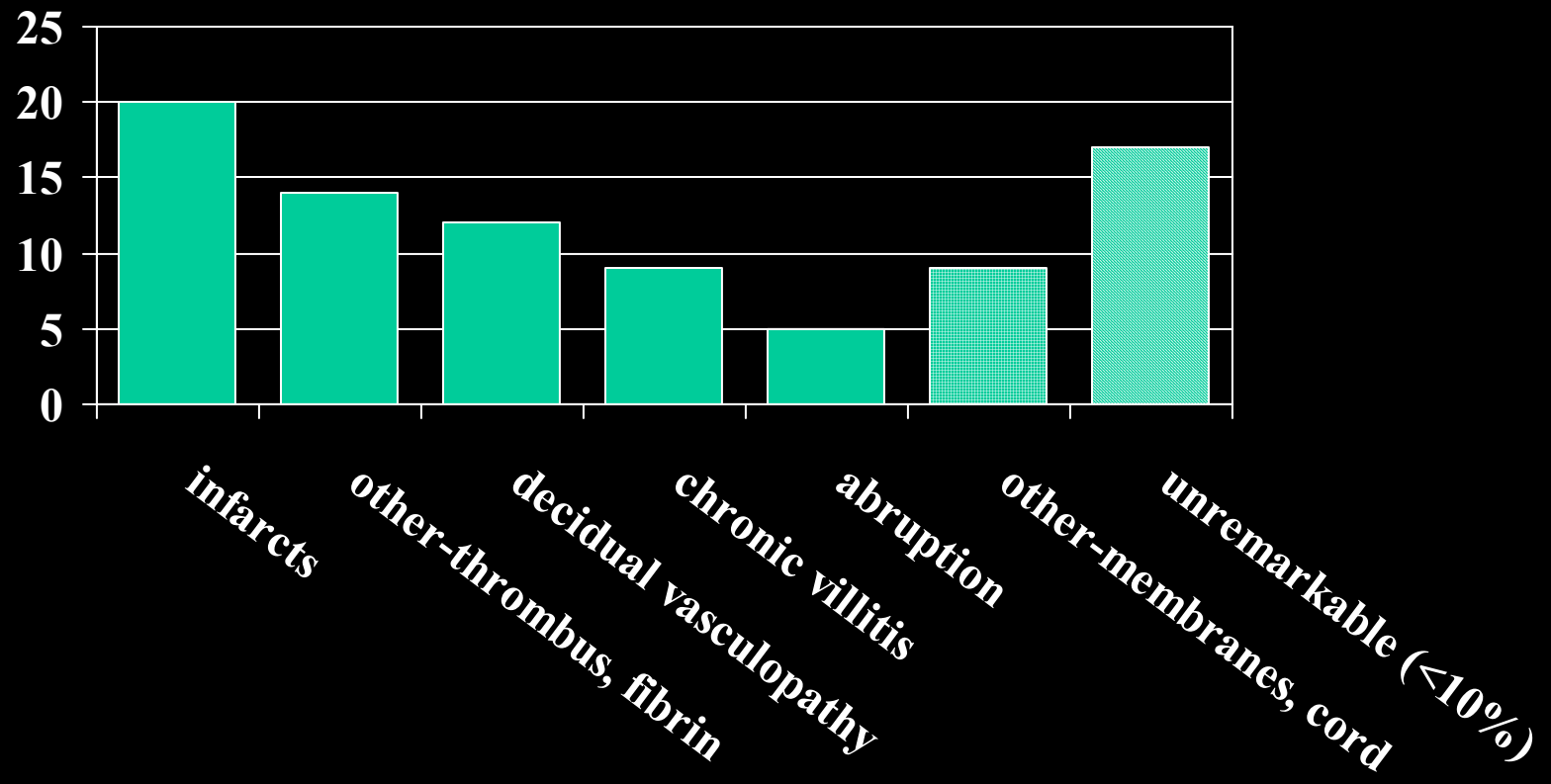
Birth Weight Distribution - Males



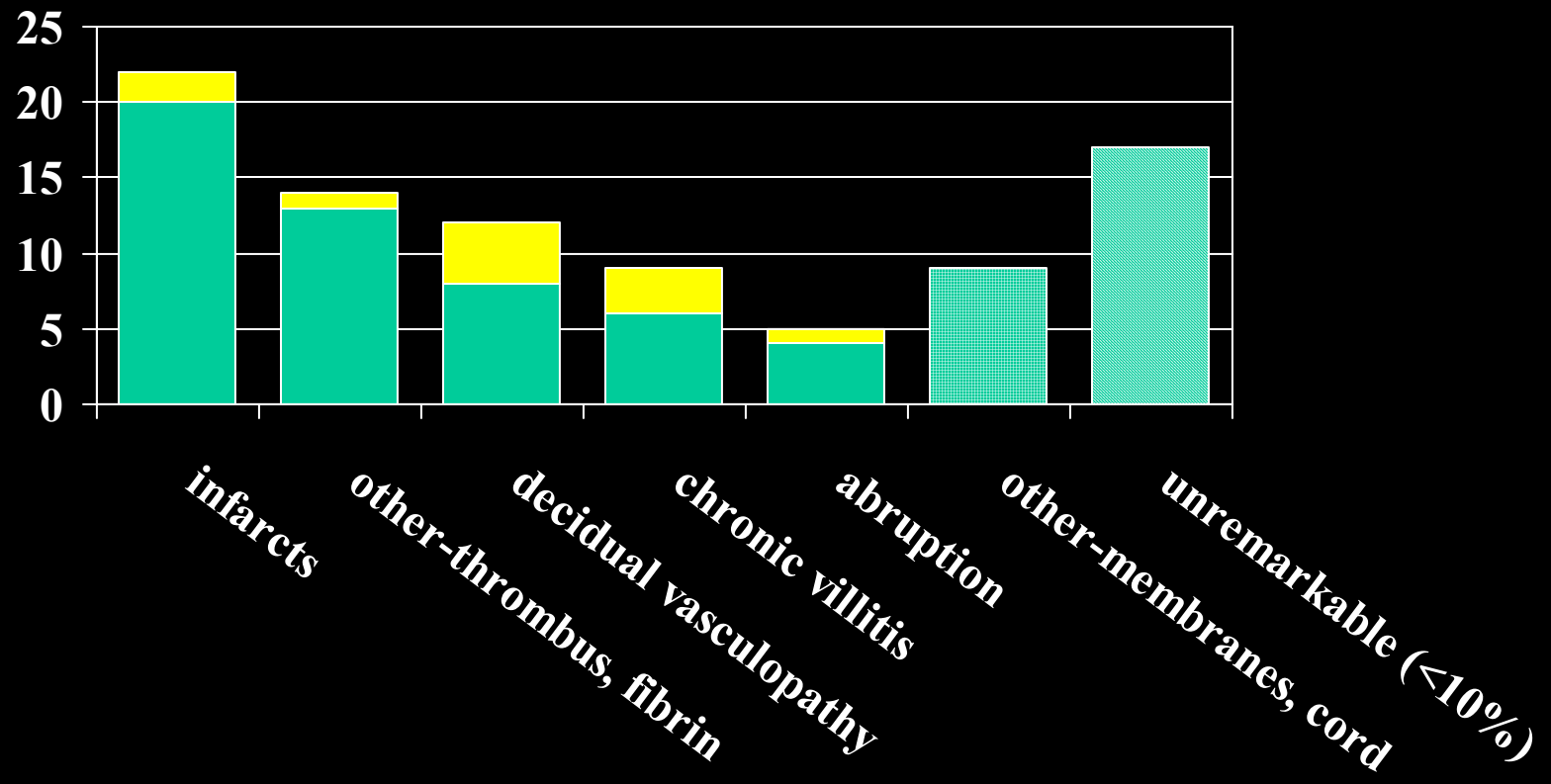
Birth weight Distribution - Females



Placental Histology



Placental Histology



Results by molecular testing – UPD among placentas with normal karyotype

- 16 sets of mother / father/ newborn DNA extracted
- All autosomes examined with 1 to 3 dinucleotide repeats
- End point of confirming biparental

Confirming biparental

- I – infant's polymorphisms only consistent with biparental
- II – consistent with both biparental and uniparental
- III – only consistent with uniparental

Analyses Performed

- Type I 352 markers
- Type II 704 markers
  - Resolved as biparental on subsequent analyses (additional 1 or 2 markers per chromosome)
- Type III 5 markers

UPD Results

- Maternal heterodisomy chromosome 14
- Paternal isodisomy chromosome 9
- Nonpaternity

Case # 235

- Chromosome 14S617
  - M 163.1 167.3
  - F 163.1 167.1
  - B 163.1 167.3 M or F M
    biparental or maternal heterodisomy
- Chromosome 14S587
  - M 250.5 261.9
  - F 262.1 265.8
  - B 250.7 261.9 M M
    maternal heterodisomy
- Chromosome 14S308
  - M 201.0 205.1
  - F 204.8 204.8
  - B 201.0 205.0 M M
    maternal heterodisomy

Case # 236

- Chromosome 9S930
  - M 289.9 289.9
  - F 290.5 298.4
  - B 290.7 290.7 F F
    - paternal isodisomy
- Chromosome 9S921
  - M 174.6 174.6
  - F 196.5 200.60
  - B 200.6 200.6
    - paternal isodisomy F F
- Chromsome 9S921
  - M 175.0 175.0
  - F 197.0 201.1
  - B 201.1 201.1 F F
    - paternal isodisomy

Clinical Outcomes with UPD

- Maternal chromosome 14
  - 38 week infant at 2200 grams
  - Placenta notable for infarcts, villitis
- Paternal chromosome 9
  - 29 week infant at 660 grams
  - Placenta notable for infarcts

Conclusions

- CPM in 6/75 (8.0 %) well defined IUGR infants versus 1/75 (1.3 %) controls
- No consistent clinical characterization of antepartum complications or placental pathology
- UPD either itself or as a reflection of hidden CPM may play a minor role among infants with IUGR